

The entrance dynamics of polymers into a gel

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We use computer simulations to study the (driven) dynamics of a charged polymer in a disordered medium, thus mimicking the setting used in gel electrophoresis. In agreement with experiments, we find that inside the gel the mobility of the polymer is only a weak function of its length N . In contrast to this, the mean entrance time into the gel, is a very strong function of N , $\langle \tau_e \rangle \propto N^{1.3}$, and does not show any sign of saturation with increasing N . We rationalize this effect by means of a simple model and propose an experimental setup that should allow to increase the separation ability of gel electrophoresis significantly.

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Understanding the dynamics of macromolecules in disordered porous media is important in many situations of practical interest such as filtration, oil recovery, transport through membranes, and gel electrophoresis [1–6], the problem we treat in this letter. Gel electrophoresis is a simple but powerful technique used to separate polyelectrolyte strands with different length. One of its main fields of application is molecular biology, where it is used to sequence DNA. In its standard setting, fragments of various lengths are injected in a thin layer of gel, composed by, e.g., agarose powder and a buffer solution. The latter imparts a charge to each base-pair. If an external electric field is applied, the fragments migrate in its direction with shorter strands moving faster than longer ones. This, in principle, allows one to separate polymers with different length N . However, for large N the velocity depends only weakly on N and therefore it is not feasible to use gel electrophoresis as an efficient separation technique for long molecules.

In the usual experimental setup, the molecules are driven through the interface between the buffer solution and the gel by the external field. Very recently this process has called the attention of experimentalists who tried to determine if it can be used as a method to improve the efficiency of separation.

Using fluorescence microscopy, Oana *et al.* [7] concluded that DNA molecules stay at the interface for a time that decreases with increasing pore size. Unfortunately, due to the difficulty with this technique to investigate many such entrance events, it is hard to extract quantitative information, such as the dependence of the delay time on the size of the molecules or the applied field. In addition, the limited spatial resolution of $0.1\mu\text{m}$ prevents a detailed knowledge of what the polyelectrolytes actually do at the interface.

Klepárník *et al.* [8,9] investigated the injection process by measuring the time that a polymer needs to propagate a macroscopic distance. From its extrapolation

to vanishing distance, they inferred that the molecules are stuck at the interface during an interval that increases with length and speculated that it will grow with decreasing field. They attributed the delay to an electro-osmotic flow in the opposite direction of the field which hinders some of the molecules from entering the gel.

In the past, numerical simulations have proven to be a very useful tool to understand the motion of polymers in disordered media [2]. In the context of electrophoresis, some of the most significant results are the ones by Deutsch *et al.* [10–12] who solved numerically the Langevin dynamics of a driven chain moving in a two dimensional random array of obstacles, allowing, however, the self-intersection of the polymer. For rather strong fields, the numerical results showed the existence of U-like conformations inside the bulk of the gel, a result which was later confirmed experimentally by video microscopy [13].

Here we study the details of the entrance process of charged polymers into the gel by means of simulations. In particular we analyze how the time it takes the polymer to enter depends on its size and the strength of the field. Since simulations are not restricted by any resolution limit, they allow us to identify the conformations of the molecule during the entrance in the gel, which in turn will help us to understand the details of this process. By using the gathered information, we propose a setup that should significantly improve the separation-efficiency in constant field electrophoresis.

The polymer is modeled by a chain of N beads connected to each other by an unbreakable spring [14]. The interaction between two monomers i and j , that are a distance r_{ij} apart, is

$$V(r_{ij}) = 4\epsilon [(\sigma/r_{ij})^{12} - (\sigma/r_{ij})^6] - K_0 R_0^2/2 \ln[1 - (r_{ij}/R_0)^2] (\delta_{j,i+1} + \delta_{j,i-1}), \quad (1)$$

where $\delta_{i,j}$ is the Kronecker δ . The first term is strongly repulsive at short distances and represents the excluded volume. For computational efficiency it is truncated and shifted at the location of its minimum, $r_m = 2^{1/6}\sigma$. The second term represents the bond between neighboring monomers. In the following we use reduced units and measure length, energy and time in units of σ , ϵ , and $\sqrt{\sigma m/\epsilon}$, where m is the mass of one monomer and the Boltzmann constant k_B is set to 1. The constants K_0 and R_0 are 30 and 1.5, respectively. We mimic the charging effect by assigning a charge $q = -1$ to each monomer. (Note that we neglect the Coulomb interaction between monomers since it can be assumed that the solvent leads to strong screening.) If we assume that the polymer is a DNA strand with charge $\sim 150e \sim 150 \times 1.6 \times 10^{-19}C$ per base pair, these numbers translate into physical units of around 200\AA for the unit of length and 2500 V/cm for the unit of field. The gel is modeled by a disordered 2d array of point particles obtained by equilibrating a system of soft disks interacting via a r^{-12} potential at $T = 1$ [15]. A snapshot of this system was expanded by a factor of 5 and the resulting, *permanently fixed*, configuration with a liquid-like structure at short range, is our 2d gel. The typical distance between neighboring particles is ~ 7 which gives pore sizes of the order of $\sim 5 - 15$. The size of the gel perpendicular to the field is 160, and is sufficiently large to avoid finite size effects if periodic boundary conditions are used. The interaction of the polymer with the gel is given by a truncated and shifted Lennard-Jones potential with the form of the first term in Eq. (1). The solvent imparts a stochastic force on each monomer, that is mimicked by substituting after each time step the velocities of the monomers by new

ones drawn from a Maxwell-distribution at $T = 1$. The positions of the monomers are then updated by means of the velocity version of the Verlet algorithm, using a time-step = 0.01.

At the beginning of each simulation we equilibrate the polymer by taking into account only the monomer-monomer interactions. Typically, the polymer acquires an open random-walk like conformation with a Kuhn length $b \sim 2 - 5$ (estimated from visual inspection), which is of the order of the pore size and corresponds to $400 - 1000\text{\AA}$. Once the polymer is equilibrated we slide it in the direction of the field until it is at a distance r_m from the closest particle in the gel. This instant defines the initial time $t = 0$ when the polymer starts interacting with the gel. We then turn on the field, driving the polymer into the gel.

We have studied polymer sizes $N = 25, 50, 100, 200$, and 400 and fields $E = 0.0312, 0.0625, 0.125, 0.25, 0.5$, and 1.0 . In all runs the temperature is such that $k_B T = 1$, which we identify with room temperature $T = 300K$. This leads to an adimensional parameter $qEb/k_B T$ between 0.1 and 10 . To improve the statistics we average all results over at least 30 independent runs.

One possibility to characterize the dynamics *inside* the gel is to monitor the time dependence of the averaged center-of-mass position in the direction of the applied field, $y_{cm}(t)$, shown in Fig. 1 for various N . After a transient, the curves become straight lines with slope $v(t)$. In Fig. 2 we plot the mobility at large times, $\mu_\infty = \lim_{t \rightarrow \infty} v(t)/E$, as a function of N . We see that to a good approximation μ_∞ is independent of E and that it is only a weak function of N . (A change of N by a factor of 100 changes μ_∞ only by a factor of less than two.) The reason for this is that for long polymers not only the force due to the field is proportional to N but also the effective friction due to the gel is linear in N . For large N the two forces cancel and the resulting velocity has only a weak N -dependence [3–5]. Therefore it is very hard to separate strands with similar lengths.

From Fig. 1 we also recognize that at short times the mean velocities have a stronger N -dependence than at long times, when the polymer is in the bulk. In Fig. 3 we show a series of snapshots that help us understanding this result. At early times, Fig. 3a, the field drives the (relatively open) equilibrium configuration in an unhindered fall towards the gel. The polymer hits the gel and its mobility decreases slightly. After a short interval some monomers, either at the end or in the middle of the polymer, find their way into the pores while others are still pinned at the boundary, Fig. 3b. The configuration acquires little hernias that penetrate the gel, Fig. 3c. Subsequently, the little hernias are eaten up by the longer ones [16] and a “staple-like” configuration is reached with two elongated arms that penetrate the gel, Fig. 3d [18]. For much longer times the longer arm will finally drag the polymer into the gel. The transient dynamics due to the entering process has ended and the asymptotic mean velocity is reached. For large N , the time it takes to resolve this staple configuration is determined by the difference in length between the arms. Since this time is significantly longer than the time it takes the polymer to reach this configuration, it determines the entrance delay almost completely.

We mention that such staple-like configurations also occur *inside the gel*, though with two important differences. i) Unless the field is extremely weak, their width is generally smaller, since the pinning is due to only one or two obstacles. In the entering case instead, the pinning occurs at more sites (therefore we call the config-

uration “staple-like” and not hernias) since outside the gel the polymer is open with a typical cross-section that scales like the radius of gyration. ii) Inside the gel the generation of such pinned configurations is a *non-synchronized* stochastic process. At the interface most strands are trapped almost immediately after touching the gel and hence the trapping is *synchronized*. These two points explain why the motion of the polymers close to the surface is so much slower than inside the gel. We also mention that none of the polymers we generated bounced back into the solvent when they encountered the interface, i.e. moved against the direction of the field. This shows that there can be a significant delay also without an electro-osmotic flow, in contrast to the explanation put forward by Klepářník *et al.* [8,9] of their experimental results.

In order to analyze how the entrance time depends on length and field, we define a time τ_e such that $y_{cm}(\tau_e) = \alpha N$, with $\alpha = 0.75$. (The precise value of α is irrelevant as long as the probability for the polymer to have entered more than αN without having been entangled is very small.) In Fig. 4 we show $\langle \tau_e \rangle$ multiplied by E versus N for different values of E . From this plot we conclude that the entering time is inversely proportional to the field. Much more important, however, is the strong dependence of $\langle \tau_e \rangle$ on N (note the logarithmic scale on the $\langle \tau_e \rangle$ axis!). This dependence does not saturate with N and it is approximately given by the law $N^{1.3}$ (bold solid line). Therefore it should be possible to use this entering process as a tool to increase the selectivity of gel electrophoresis.

A very simple model allows us to understand on a qualitative basis the origin of this strong N -dependence: Let us assume, as suggested by the simulations, that we can neglect the time needed to reach the staple-like conformation and hence can approximate τ_e by the time necessary to resolve it. The problem can now be modelled by a massive rope with uniform linear density q that hangs under gravity, with strength E , from a pulley and whose motion is slowed down due to friction that is proportional to its velocity. The arm carrying more mass makes the rope slide in its direction. If we call δ the difference in length of the two arms, normalized by the total length of the rope, it is easy to calculate the time needed for the rope to fall from the pulley and one finds

$$\tau_e = -\frac{\xi N}{2qE} \ln \delta \quad , \quad (2)$$

where ξ is the friction constant per unit length. In our problem δ fluctuates from sample to sample. Therefore an average over samples translates into an average over δ . If we assume that the distribution of δ is independent of δ we obtain $\langle \tau_e \rangle \propto \xi N/E$. The stronger N -dependence shown in Fig. 4 can be accounted for by assuming that the distribution of δ has a somewhat larger weight at small δ .

Equipped with the results of the simulation we propose an experimental setup that should allow to increase the ability of gel electrophoresis to separate polymers of different length. In this setup we have an alternating sequence of strips made out of gel and strips with just solvent. If the field is pointing upwards, these strips are arranged vertically and have “heights” g_1, s_1, g_2, s_2, g_3 , etc. The basic idea is as follows. If the polymer mixture is placed below the first strip all polymers enter this strip, leading to the very strong separation studied here. The polymers later leave the first strip and enter a strip of solvent. The polymer changes its conformation

from the elongated structure acquired in the gel to a random walk-like structure since in the pure solvent the elongated structure is not stable. After some time it hits the next solvent-gel interface and the process starts again. In order to allow a strong separability at the interface, the width of the gel has to be at least on the order of the longest polymers. To ensure that long polymers open up again during their fall in the solvent, this strip also needs to be sufficiently long. Therefore it is advisable to increase progressively the width of the strips i.e. $g_1 < g_2 < g_3 < \dots$ and $s_1 < s_2 < s_3 \dots$

We conclude by stating that these results, together with the ones in Refs. [7–9], make us expect that the use of interfaces can lead to a strong improvement of the separability in gel electrophoresis.

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with two elongated arms, or only one end and the middle section of the polymer enter, giving rise to an elongated arm on one end and a double segment at the other end. If this double arm has more mass than the elongated one, it will grow, the polymer will acquire the staple-like configuration, and it will eventually pull the polymer inside the gel.

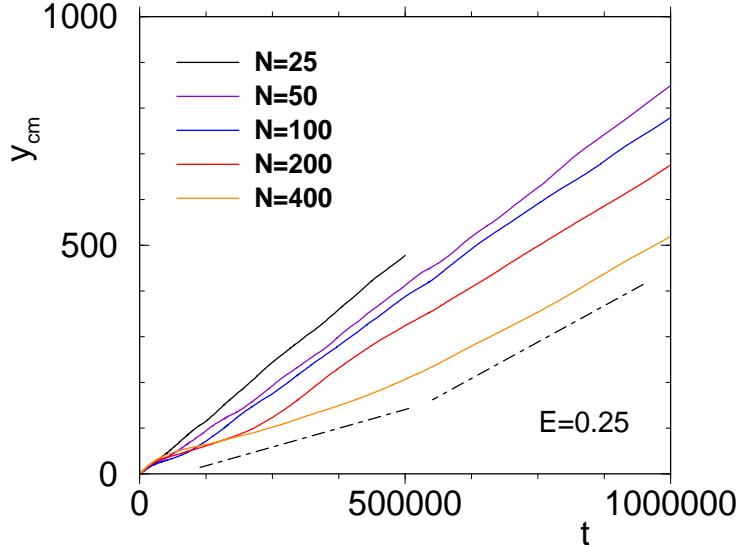


FIG. 1. Time dependence of the mean center-of-mass position in the direction of the field for different polymer lengths. There are two time regimes for the long polymers, indicated by the two dashed-dotted lines.

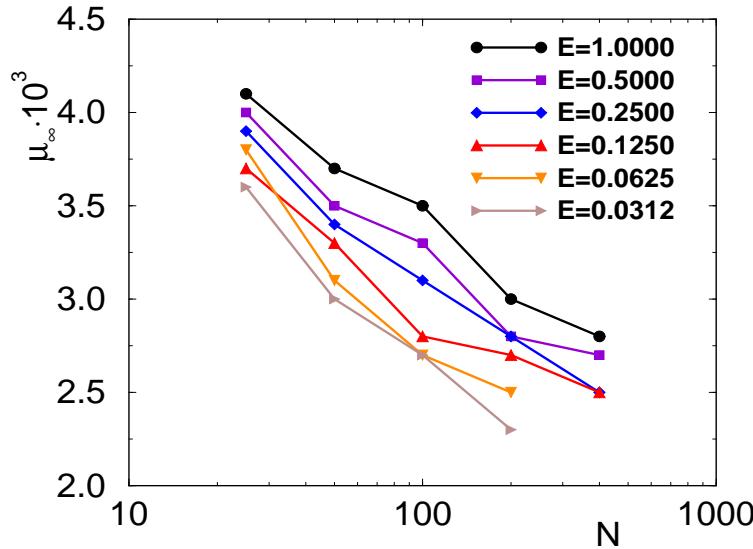


FIG. 2. Size-dependence of the mobility at long times for different external fields.

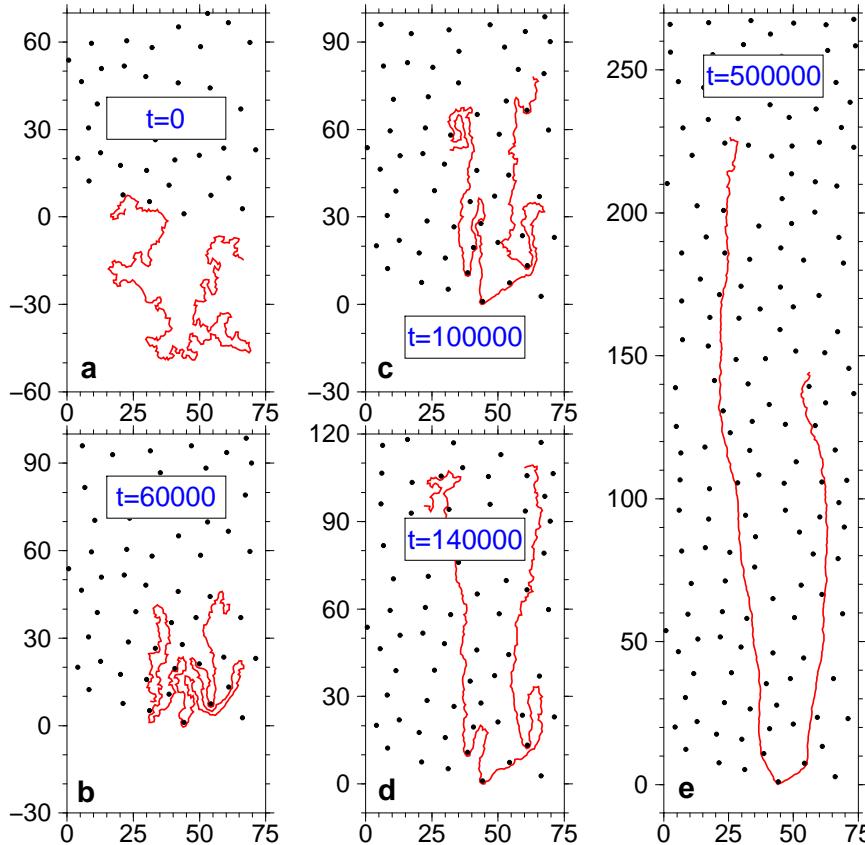


FIG. 3. Snapshots of the polymer during the entering process into the gel. The black circles represent the obstacles in the gel and their size corresponds, approximately, to the range of the interaction. The external field is pointing upwards. $N = 400$ and $E = 0.25$.

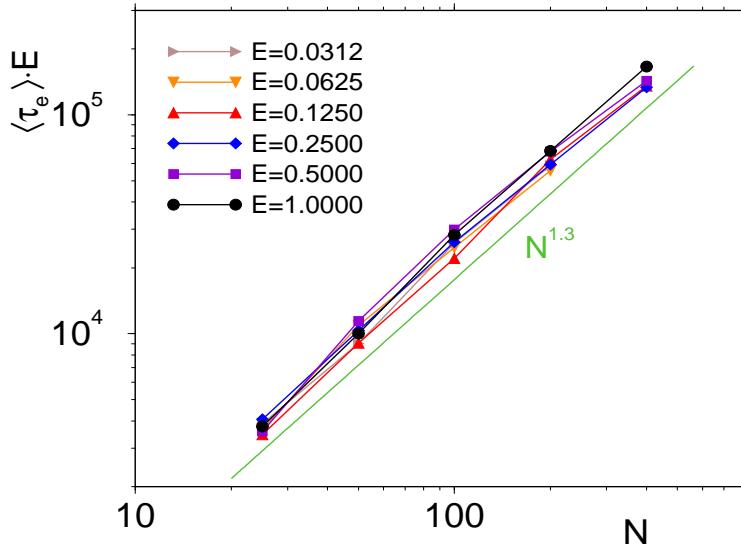


FIG. 4. Scaled entering time $\langle \tau_e \rangle E$ as a function of the polymer length for various values of the field E .